fauna in the "Temblor" beds of the North Coalinga region, it seems clear that these marine beds, commonly referred to Lower Miocene or late Oligocene, are not older than mammal-bearing beds of the interior of the continent referred to Middle Miocene.

The "Temblor" beds of southern California represent a phase of the Monterey series of California, which is one of the best known and most widely spread of the divisions of the Tertiary. There seems good reason to believe that the Monterey series of California is approximately to be correlated with the Mascall Middle Miocene of the Great Basin.

A broad consideration of the lack of adjustment between the time scale of the Pacific Coast province and that of the Great Basin suggests that correlations of marine faunas of the Pacific Coast region, particularly those based on the percentage method, have tended to locate the time divisions relatively too far from the present or Recent. In late years, the refinement of specific characterization has proceeded very rapidly. Splitting the species has resulted in giving us a larger number of forms each of which has a relatively restricted geographic and geologic range. The percentage method, as proposed by Lyell, when used with modern species naturally results in pushing time divisions farther apart.

The lack of adjustment in the time scale also suggests the desirability of testing the relation of Middle Miocene mammal-bearing beds of North America to the formations of Lower Miocene age in the European scale.

The fauna of the second mammal zone of the Coalinga region comes from beds referred for the present to the Jacalitos formation. It includes a form referred to *Protohippus* by Arnold and Anderson, and a *Neohipparion* species of somewhat advanced stage. The *Neohipparion* material from this zone is insufficient for thoroughly satisfactory comparison. It seems in part to be related to a *Neohipparion* from the Rattlesnake Pliocene of the John Day region of eastern Oregon. This species does not appear to be very closely related to the well-known *Hipparion* species in the Ricardo fauna from the Mohave Desert.

The fauna of the third or Protohippus coalingensis zone of the Etchegoin formation in the Coalinga region has as its most characteristic form a new species, *Protohippus coalingensis*,[1] which differs from all the described species found west of the Wasatch Range. It is most nearly related to a species represented in the Ricardo fauna of the Mohave Desert. It does not, however, seem to be identical with the Ricardo form. The stage of this fauna, in very general terms, seems to be Pliocene. Both the Etchegoin of this zone and the Jacalitos below it were referred by Arnold and Anderson[2] to the Upper Miocene.

The fourth fauna of the North Coalinga region includes a number of species of relatively modern aspect. These include forms referable to *Equus* and to *Cervus* or *Odocoileus*. This assemblage may be known for the present as the Equus-Cervus fauna. Its stratigraphic position is not entirely clear. The fauna is in part much like that of the Pleistocene.

JOHN C. MERRIAM

THE CRENATION AND FLAGELLATION OF HUMAN ERYTHROCYTES

I. *Crenation*

THE method of preparing the blood on which the following observations on crenation were made is very simple. A drop of blood obtained by pricking the finger is immediately sucked up into a pipette which contains one to two cubic centimeters of sterile Ringer's solution or 0.85 per cent. sterile sodium chloride or human blood serum. The suspension is then mixed on a sterile glass slide until a homogeneous suspension is obtained. A drop of the suspension is then transferred by means of a pipette to an absolutely clean large coverslip and the drop allowed to spread out into a thin

[1] *Protohippus coalingensis*, n. sp. Type specimen, No. 21,341, Univ. Cal. Col. Vert. Palæ. Distinguished by large size, unusual narrowness of cheek-teeth in transverse diameter, small protocone and narrow, simple fossettes.

[2] Arnold, R., and Anderson, R., U. S. Geol. Surv. Bull. No. 398, p. 78, 1910.

film. The preparation is then mounted in a glass moist chamber, open to the air at one end, and examined at ordinary room temperature. A drop of untreated human blood mounted in a moist chamber serves equally well, if the corpuscles be more or less separated, by spreading the blood into a thin film on the cover-slip.

The preparations were studied at room temperature by means of both natural and artificial light. A frosted Mazda light globe of sixty watts was used as the source of illumination, the light being passed through a glass container containing sufficient copper chloride to impart a weak blue color to the solution. The following observations were made with an ordinary Leitz 1:12 oil immersion lens and a No. 4 ocular. Certain finer details of structure were better revealed by a No. 12 compensating ocular.

The microdissection technique used is the same as that employed by Kite[1] and involves the use of the Barbour pipette holder, the Barbour moist chamber, and exceedingly fine (1–2 microns) hard Jena glass needles and pipettes.

When blood is prepared as above described certain of the cells are seen to have undergone more or less pronounced crenation as soon as they can be examined. If now a very fine needle point be brought up under a crenated erythrocyte, then carefully elevated so as to just touch the cell, and then immediately lowered, the corpuscle instantly regains an optically normal appearance and retains it for hours. Crenated cells thus brought back to the normal have never been seen to undergo subsequent crenation if left undisturbed. (It should be noted that in bringing the fine point of the dissecting needle into contact with the cell extreme care must be taken; otherwise, although the cell immediately rounds up and swells, yet within 20 to 30 seconds the hæmoglobin dissolves out and only a so-called "shadow corpuscle" remains.)

If a fine needle be raised into a drop containing normal red blood cells no crenation occurs when the needle pierces the meniscus of the drop. If now the needle point be brought up alongside the cell (not touching the cell but in the same focal plane) the corpuscle immediately crenates. The amount of crenation seems to be dependent somewhat on the proximity of the needle to the cell. As long as the needle remains in place the crenation persists, but as soon as the needle is lowered out of the focal plane of the cell the corpuscle instantly goes back to the normal. This experiment of crenating and uncrenating a cell can be indulged in indefinitely, with always the same results.

Various methods were employed. If, for instance, a fine microdissection needle be brought up alongside a completely crenated cell, and if the needle point be then carefully moved against the cell, pushing in a small arc of the cell substance before it, immediately on lowering the needle away from the cell, the corpuscle rounds up and swells. In all the above-quoted and subsequent experiments cells brought back from the crenated stage remained intact and optically normal. In fact, such cells can not be distinguished from absolutely normal red-blood cells.

Even more striking results on a somewhat larger scale are obtained when, instead of a needle, a very fine pipette is employed. The best results are obtained with a glass pipette whose lumen is not more than one micron in diameter. If such a pipette be raised into a field of crenated erythrocytes the instant the pipette pierces the meniscus of the drop all of the crenated and otherwise distorted cells in the field immediately round up and retain their perfectly normal, regular outline and appearance so long as the pipette is allowed to remain in the drop. If now the pipette be lowered out of the drop, all the cells immediately go back to their irregular, crenated shape. The cells that were originally of a pointed oval shape, etc., for instance, return to their oval form, and the variously crenated cells return to their original stage of crenation.

If, into a drop containing perfectly normal red-blood cells, a very fine pipette is raised and the experimenter exerts a very slight suction

[1] A detailed description of the method will be published shortly.

on the pipette, all the cells within a more or less definite zone about the pipette instantly crenate. If now the experimenter blows into the pipette very slightly (the pipette, of course, still being in the drop) the cells immediately round up and remain perfectly normal. This alternate crenating and uncrenating the cells can be indulged in repeatedly.

Examination of red-blood cells kept for hours in a moist chamber gives evidence that probably there are a number of more or less definite types and stages of crenation. In preparations of crenated erythrocytes a varying number (dependent somewhat upon the age of the preparation) are seen to undergo an internal change (as noted by Kite) which is characterized by the formation of refractile granules and rods, of somewhat definite size, in the cell substance. The exact relation of this phase to crenation has not yet been determined. The deposition of these rods and granules is very possibly a coagulation phenomenon. Cells that have undergone such a change are apparently more stable and less easily brought back to the normal than crenated but optically homogeneous corpuscles. Such cells can be sucked up into a pipette and expelled into the same or different drop without undergoing any apparent alteration in shape or size. Such a cell can, however, be brought back to the normal by raising a needle against the cell body and immediately lowering it. The granules and rods instantly disappear and the cell immediately assumes an apparently permanent normal outline and appearance.

All of the above experiments can be performed equally well whether the blood cells be mounted in an isotonic, slightly hypotonic or slightly hypertonic solution. Certain of the experiments, especially, would seem to indicate that the phenomenon is apparently outside the sphere of any possible osmotic process, dependent upon an alteration in a hypothetical semipermeable membrane around the red-blood corpuscle. Rather the experiments would lead one to suspect that the shape a red-blood cell assumes is an expression of surface tension forces. The experiments also serve to emphasize the extreme irritability of protoplasm.

II. *Flagellation*

In an article[2] to be published shortly in the *Journal of Infectious Diseases,* Kite records a series of dark-field observations on the structural modifications undergone by the blood cells of various vertebrates when mounted in liquid plasma containing Ringer's fluid and hirudin and examined in sealed preparations. He records dark field observations of various types of both motile and non-motile processes which appear on the blood cells of vertebrates.

After studying certain of these structural changes in sealed preparations by means of the dark field and special condensers it seemed of interest to more carefully study red-blood cells mounted in a Barbour moist chamber freely open to the air, and to determine whether these changes could be seen by ordinary transmitted light and without the aid of special condensers. For this purpose the following experiments were undertaken. It should be recorded here that, although one type of process mentioned below is apparently coarser and of a somewhat different nature than any of the processes figured by Kite, yet there is no reason to suppose that this type of process is anything more than possibly another phase in the transformations described by him. As can be determined by reference to Kite's paper, priority of certain of the following observations made under somewhat different conditions belong to him. Control observations with hirudinized preparations have been made with the same results. The method of preparing the microscopic mounts is the same as described above under crenation.

Immediately upon making the preparation a large proportion of the red-blood cells are seen to possess very short non-motile spinous processes which line the entire periphery of the cell. Within forty to fifty minutes after the preparation is made the erythrocytes are seen to possess long processes, some of which exhibit a rapid whip-like motion, others a slow undu-

[2] "Some Structural Modifications of the Blood Cells of Vertebrates," G. L. Kite. Read before the Society for Experimental Biology and Medicine, April 15, 1914.

latory movement, while still others are absolutely motionless. These processes, which can be seen to be thrown out from the cell and possess unquestioned continuity with the cell, apparently originate from small blunt projections which appear on the surface of the cell. These processes appear alike on crenated and uncrenated cells, are comparatively easily seen, and vary in length from two to three microns to as long as 30 microns. Under certain conditions, the details of which have not been worked out, they are capable of extremely rapid retraction. Frequently oval erythrocytes with the two ends drawn out into a long fine whipping process which may have a length of five to six times the diameter of the cell are found. Cells with these beating processes are rapidly whipped across the field. These long fine processes, when they first appear on the red cells, are of a clear, non-granular nature. After whipping for twenty to thirty minutes they have been seen to take on a granular, beaded appearance. The beaded processes continue to beat. If watched, certain of these processes can be seen to break off from the cell, and even after being detached, continue to whip across the field. If these detached processes are further observed they can be seen to eventually break down, the granules floating free in the preparation and exhibiting marked Brownian movements. These granules apparently hold up, and at the end of five or six hours are found in large numbers.

By means of the microdissection technique devised by Kite the fine beating processes on the red blood cells have been dissected off. When a process is dissected off the cell, the broken-off process may remain sticking to the point of the needle. The free end continues to whip for as long as forty minutes after being detached from the cell. If a process be dissected off near the cell the small portion remaining attached to the cell continues to whip.

If a motionless process on an erythrocyte is touched at any point along its extent by a very fine needle the process immediately begins to whip. For instance, an erythrocyte of perfectly regular outline with a long (20–30 micron) process at each pole of the cell was watched for forty-five minutes. During this time the cell did not change in outline, and the cell and its processes remained absolutely motionless. At the end of this time one of the processes was touched near its base. The process immediately commenced to whip, and the motionless process at the other pole of the cell took on a very slow undulatory movement. When this latter undulating process was touched by the needle, it, too, immediately commenced to whip. The two actively whipping processes soon carried the cell out of the field, and the cell was followed in its progress through a number of fields. At the end of thirty minutes the processes were still whipping the cell through the preparation. The long processes are exceedingly flexible and seem to beat in an arhythmic manner. They frequently are seen to whip around the cell to which they are attached, and become glued to the surface of the cell. After several minutes they can be seen to beat free from the cell and continue their active whipping motion. The apparent viscidity of the processes is evidenced by the fact that two or more beating processes of the same or neighboring cell frequently become entangled and stick together. They may become freed naturally, or they can be pulled apart by means of the dissecting needle. At times the middle portion of a long process becomes stuck to the cell while the free terminal portion continues to whip.

If a dissecting needle be brought up along side the middle portion of one of these long beating processes, and this portion then be carefully pushed so as to form an arc, the distal portion of the process continues to beat in a line with the motionless proximal portion. If too much tension is placed on the process it is torn loose. The various types of motile and non-motile processes on the red-blood cells can be found in moist chamber preparations of blood mounted in 0.85 per cent. sodium chloride for many hours after the preparation is made (at least twenty-four hours).

Wade W. Oliver

Marine Biological Laboratory,
Woods Hole